PDE2 inhibitor | BI-1960

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Summary

BI-1960 is PDE2 inhibitor with a good *in vitro* and *in vivo* potency, good brain penetration, and an acceptable PK profile. The compound has shown central functional target engagement and pro-cognitive efficacy in rodents. It has a remarkable selectivity and good *in vivo* profile with high bioavailability in mice and rats.

Chemical Structure

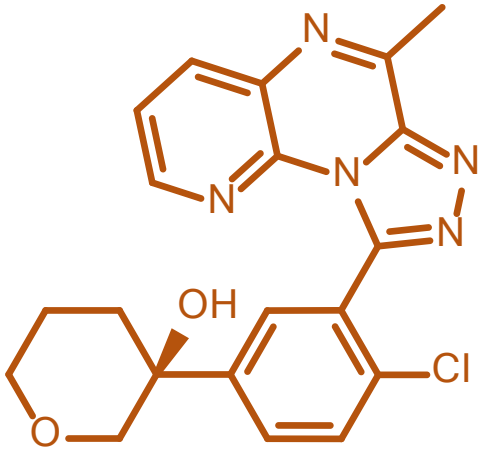

Figure 1: 2-D structure of BI-1960, a potent and selective PDE2 inhibitor

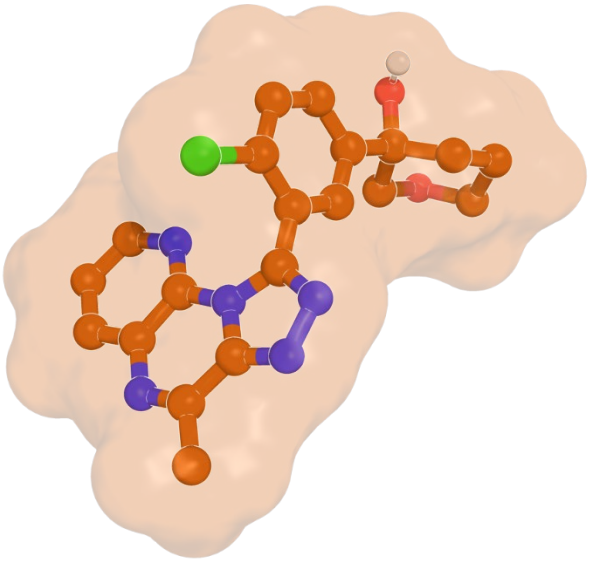

Figure 2: Low energy 3D structure of BI-1960

Highlights

BI-1960 is a small molecule PDE2 inhibitor with a *good in* vitro and *in vivo* activity, good brain penetration, and an acceptable PK profile. This compound has a remarkable selectivity and good *in vivo* profile, allowing for an oral application with high bioavailability in mice and rats. The structurally related BI-4392 can be used as a suitable negative control.

Target information

Cyclic nucleotide degrading phosphodiesterases (PDEs) are a superfamily of hydrolyzing enzymes that play a pivotal role in the regulation of secondary messengers 3′,5′-cyclic nucleotides cAMP and cGMP. PDE2 is a member of this broad family of PDEs of special interest due to its ability to potentiate glutamatergic transmission in brain areas involved in emotion, learning and memory such as cerebral cortex and hippocampus.[4] PDE2 was cloned and sequenced in 1986. Three different splice variants of PDE2 are encoded by a single gene (PDE2A) and differ only in the N-terminus but not in the C-terminus which includes the catalytic domain. However, no differentiating function could be associated with any of these splice variants. PDE2 is highly conserved across species. Human PDE2 protein shows 90.1 and 89.1 identity with the rat and mouse PDE2 protein, respectively (source www.uniprot.org). Protein identity in the catalytic domain (region 633-891) is >99% with respect to mouse, rat, dog, and pig. The downstream signaling pathway is also conserved across species. The inhibition of PDE2 increases the neuronal levels of cyclic nucleosides cAMP and cGMP at the pre-synaptic level in brain regions believed to be involved in learning and memory. PDE2 inhibition in the brain could improve glutamatergic neurotransmission and strengthen synaptic plasticity which according to pre-clinical evidence may have the potential to influence cognition in patients with Alzheimer's disease or Schizophrenia.[1,2,3,7]

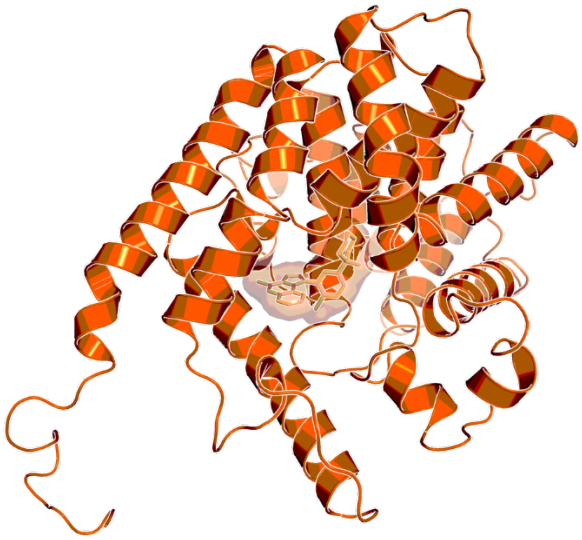

Figure 2: BI-1960 binding to the catalytic domain of PDE2; Co-crystal structure of the probe compound and the target protein

In vitro activity

BI-1960 is a potent and selective PDE2 inhibitor. Using the purified catalytic domain of human, and rat PDE2, BI-1960 exhibits an IC_{50} in the IMAP assay of 22 and 44 nM, respectively. Thus, BI-1960 does not show a relevant species selectivity across rat and human which agrees with the high amino acid sequence analysis and the binding site. No relevant selectivity issues versus other PDEs could be identified (IC_{50} > 10 µM).[5]

PROBE NAME / NEGATIVE CONTROL	BI-1960	BI-4392
MW [Da]	395.85	354.79
PDE2 IC_{50} [nM] - human [a]	22	>10,000
PDE2 IC_{50} [nM] - rat [a]	44	-
PDE10 IC_{50} [nM] – human [a]	9,654	2,140

[a] Inhibition of PDE 2A or 10 enzyme activity was assessed using IMAP-Phosphodiesterase-cAMP fluorescence labeled substrate, IMAP TR-FRET screening express kit and PDE 2A or PDE10 proteins expressed upon baculovirus infection in SF9 cells. For protein expression the cells were incubated after infection with the virus for ~3 days and protein production was confirmed by Western Blot. The cells were collected by centrifugation and the pellet frozen in liquid nitrogen before it was resuspended in PBS containing 1% Triton X-100 and protease inhibitors. After 45 min incubation on ice, the cell debris was removed by centrifugation (13.000 rpm, 30 min). Since SF 9 cells do not express cAMP hydrolyzing enzymes to a high extent, no further purification of the protein was needed. All reactions were performed in 384 well, Perkin Elmer black optiplates and IMAP reaction buffer with 0.1% Tween20 and 0,25% BSA.

In vitro DMPK and CMC parameters

BI-1960 is characterized by a high permeability and no efflux in Caco-2 cells suggesting good gastrointestinal absorption. In MDCK cells overexpressing P-gp, a moderate efflux (ER=3.0) was observed proposing a low impact of P-gp on central exposure. This was confirmed in rats and mice where a comparable moderate *in vivo* efflux was measured (muscle/brain: 3.5). Plasma protein binding is medium to low in all investigated animal species with the highest PPB in humans and Volume of distribution (V_{ss}) is in a medium range across species.

PROBE NAME	BI-1960
logP	1.8
Solubility @ pH 6.8 [µg/ml]	60
CACO permeability @ pH 7.4 [*10^{-6} cm/s]	91
CACO efflux ratio	0.7
MDCK permeability P_{app}a-b/b-a @ 10µM [10^{-6} cm/s]	52
MDCK efflux ratio	3
Microsomal stability (mouse/rat male/rat female) [% Q_H]	39/38/<22
Hepatocyte stability (human/mouse/rat) [% Q_H]	5 /64 / 54
Plasma protein binding (human/mouse/rat) [%]	16 / 30 / 48
hERG [inh. % @ 10 µM]	>10 µM
CYP 3A4 (IC_{50}) [µM]	> 50
CYP 2C8 (IC_{50}) [µM]	>50
CYP 2C9 (IC_{50}) [µM]	>50
CYP 2C19 (IC_{50}) [µM]	>50
CYP 2D6 (IC_{50}) [µM]	>50

In vivo DMPK parameters

BI-1960 possesses high bioavailability and exhibits a medium to high metabolic stability in rat hepatocytes which correlates well with the *in vivo* CL in those species. Overall, the clearance and distribution behavior of BI-1960 results in moderate MRT across species. Minor renal elimination of parent compound was observed (<2% of dose) in rats.

PROBE NAME	BI-1960
Clearance (mouse [a] /rat [b]) [% Q_H]	77 / 66
Mean residence time after or dose (mouse [a] /rat [b]) [h]	20 / 1.4
t_{max} (human) [h]	4
F (mouse [a] /rat [b]) [%]	20 / 80
V_{ss} (mouse [a] /rat [b]) [l/kg]	0.8 / 1.9

[a] Mouse dose p.o.: 10 µmol/kg

[b] Rat dose p.o.: 7.5 µmol/kg

In vivo pharmacology

To demonstrate increase in cGMP after PDE2 inhibition in animals, cGMP was measured post-mortem in different brain regions in the mice (cortex, striatum and hippocampus) via ELISA after oral administration of BI-1960. BI-1960 causes a dose-dependent significant increase in cGMP in naïve mice at 20 mg/kg, corresponding to CSF concentration exceeding 1-fold PDE2 IC_{50}.

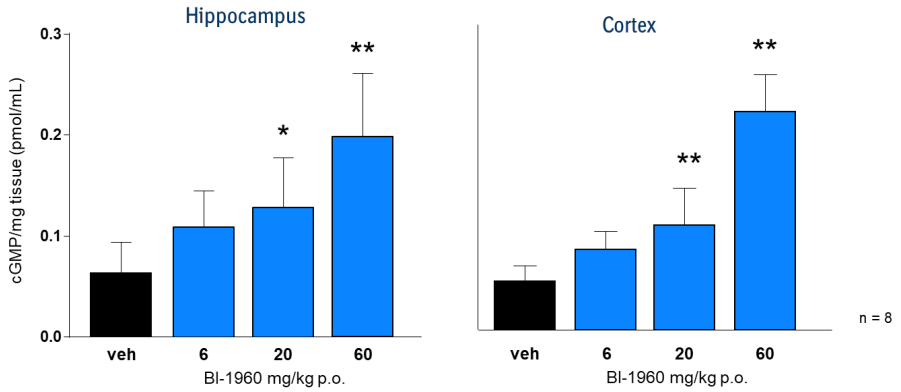

Figure 3: Effects of BI-1960 on cGMP concentration in naïve mice

In vivo efficacy could be demonstrated in two cognition tasks addressing memory domains known to be affected in schizophrenia (working memory) and Alzheimer's disease (episodic memory), the T-maze spontaneous alternation test in mice and the social recognition test in naïve rats, respectively. BI-1960 demonstrated efficacy in mouse T-maze test on reversal of MK-801[6] induced memory deficits at doses of 1–3-10-30 mg kg p.o., corresponding to compound levels covering CSF concentration of 0.6–30 x PDE2 IC_{50}. In the social recognition test in naïve rats, memory performance was improved at the dose of 1.5 mg/kg p.o. corresponding to CSF levels 1-fold PDE2 IC_{50}.

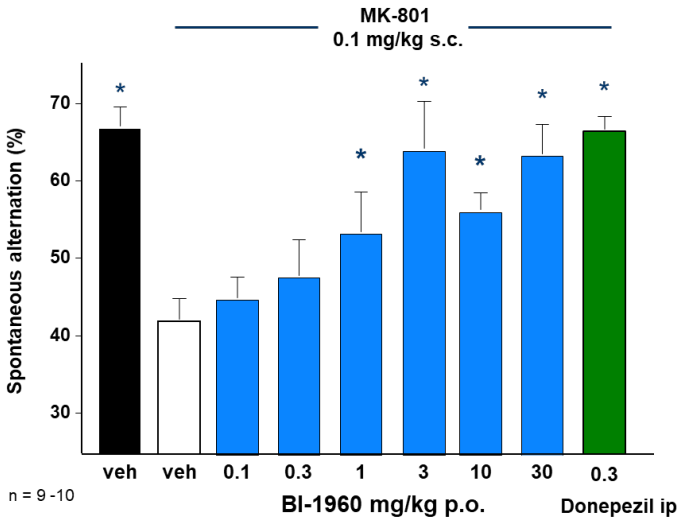

Figure 4: Effects of BI-1960 in mouse T-Maze

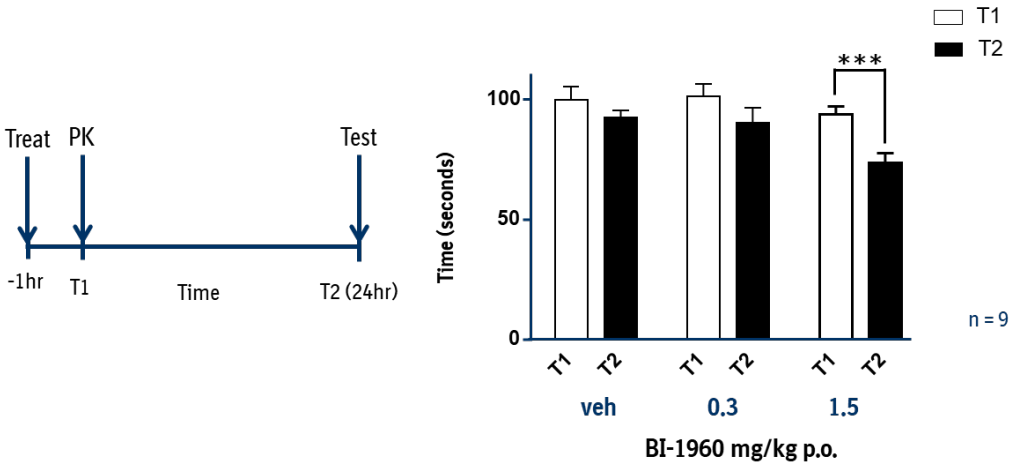

Figure 5: Effects of BI-1960 in the rat social recognition test

Negative control

The negative control BI-4392 has a similar structure to BI-1960, but it has low affinity on PDE2 in the IMAP assay.

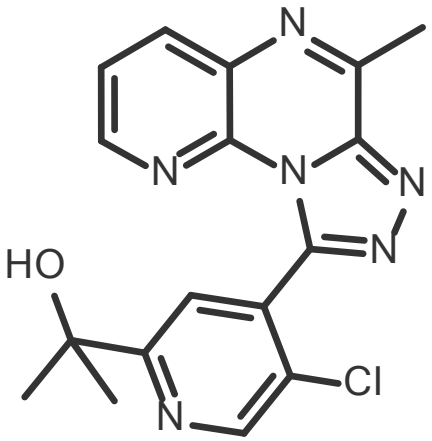

Figure 6: BI-4392 which serves as a negative control

Selectivity

BI-1960 possesses remarkable selectivity versus other PDEs (IC_{50} > 10 µM). BI-1960 was tested on 114 targets in a selectivity panel and showed ≥1,000-fold selectivity for all targets (≤ 50% inhibition @ 10 µM).

SELECTIVITY DATA AVAILABLE	BI-1960
Selectivity vs PDEs	No hit @ 10 µM
LeadProfilingScreen (69 targets)	No hit @ 10 µM
SafetyScreen44™ with kind support of eurofins	Yes
Invitrogen®	No
DiscoverX®	No
Dundee	No

Reference molecule(s)

BAY60-7550 and PF-05180999

Supplementary data

2-D structure files can be downloaded free of charge from opnMe.

References

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